8. REBUILDING THE NERVOUS SYSTEM WITH STEM CELLS

Today, most treatments for damage to the brain or spinal cord aim to relieve symptoms and limit further damage. But recent research into the regeneration mechanisms of the central nervous system, including the discovery of stem cells in the adult brain that can give rise to new neurons and neural support cells, has raised hopes that researchers can find ways to actually repair central nervous system damage. Research on stem cells in nervous system disorders is one of the few areas in which there is evidence that cell-replacement therapy can restore lost function.

STEM CELLS BRING NEW STRATEGIES FOR DEVELOPING REPLACEMENT NEURONS

Just a decade ago, neuroscience textbooks held that neurons in the adult human brain and spinal cord could not regenerate. Once dead, it was thought, central nervous system neurons were gone for good. Because rebuilding nervous tissue seemed out of the question, research focused almost entirely on therapeutic approaches to limiting further damage.

That dogma that brain tissue could not be regenerated is history. In the mid-1990s, neuroscientists learned that some parts of the adult human brain do, in fact, generate new neurons, at least under certain circumstances. Moreover, they found that the new neurons arise from "neural stem cells" in the fetal as well as the adult brain (see Chapter 4. The Adult Stem Cell). These undifferentiated cells resemble cells in a developing fetus that give rise to the brain and spinal cord. The researchers also found that these neural stem cells could generate many, if not all, types of cells found in the brain. This includes neurons—the main message carriers in the nervous system, which use long, thin projections called axons to transmit signals over long distances—as well as crucial neural-support cells called oligodendrocytes and astrocytes (see Figure 8.1. The Neuron).

The discovery of a regenerative capacity in the adult central nervous system holds out the promise that it may eventually be possible to repair damage from terrible degenerative diseases such as Parkinson's Disease and amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), as well as from brain and spinal cord injuries resulting from stroke or trauma (see Box 8.1. Early Research Shows Stem Cells Can Improve Movement in Paralyzed Mice).

Researchers are pursuing two fundamental strategies to exploit this discovery. One is to grow differentiated cells in a laboratory dish that are suitable for implantation into a patient by starting with undifferentiated neural cells. The idea is either to treat the cells in culture to nudge them toward the desired differentiated neuronal cell type before implantation, or to implant them directly and rely on signals inside the body to direct their maturation into the right kind of brain cell. A variety of stem cells might be used for this task, including so-called "neural precursor cells" that are inwardly committed to differentiating into a particular cell type but are outwardly not yet changed or pluripotent embryonic stem cells—cells derived from a very early stage human embryo that retain the capacity to become any cell type in the body and that can be maintained in culture for a very long time without differentiating.

The other repair strategy relies on finding growth hormones and other "trophic factors"—growth factors, hormones, and other signaling molecules that help cells survive and grow—that can fire up a patient's own stem cells and endogenous repair mechanisms, to allow the body to cope with damage from disease or injury. Researchers are vigorously pursuing both strategies to find therapies for central nervous system disorders that involve cell death, but a great deal more basic research must be carried out before effective new therapies emerge.

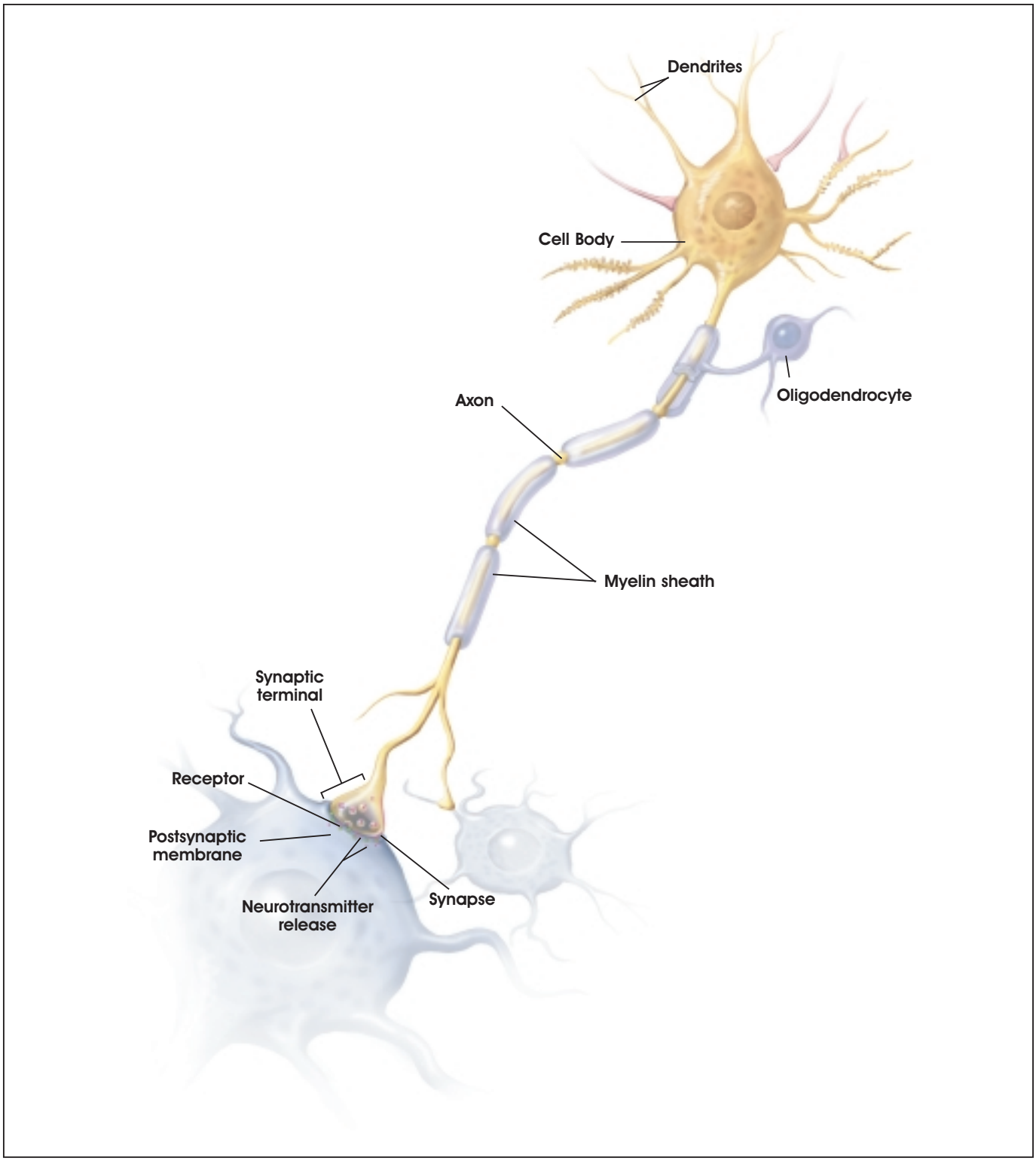


Figure 8.1. The Neuron.
When sufficient neurotransmitters cross synapses and bind receptors on the neuronal cell body and dendrites, the neuron sends an electrical signal down its axon to synaptic terminals, which in turn release neurotransmitters into the synapse that affects the following neuron. The brain neurons that die in Parkinson's Disease release the transmitter dopamine. Oligodendrocytes supply the axon with an insulating myelin sheath.

Box 8.1

Early Research Shows Stem Cells Can Improve Movement in Paralyzed Mice

Researchers at Johns Hopkins University recently reported preliminary evidence that cells derived from embryonic stem cells can restore movement in an animal model of amyotrophic lateral sclerosis (ALS) [1]. This degenerative disorder, also called as Lou Gehrig's disease, progressively destroys special nerves found in the spinal cord, known as motor neurons, that control movement. Patients with ALS develop increasing muscle weakness over months to years, which ultimately leads to paralysis and death. The cause is largely unknown, and there are no effective treatments.

In this new study, the researchers used a rat model of ALS to test for possible nerve cell- restoring properties of stem cells. The rats were exposed to Sindbis virus, which infects the central nervous system and destroys the motor neurons in the spinal cord. Rats that survive are left with paralyzed muscles in their hindquarters and weakened back limbs. Scientists assess the degree of impairment by measuring the rats' movement, quantifying electrical activity in the nerves serving the back limbs, and visually judging the extent of nerve damage through a microscope.

The researchers wanted to see whether stem cells could restore nerves and improve mobility in rats. Because scientists have had difficulty sustaining stem cell lines derived from rat embryos, the investigators conducted their experiments with embryonic germ cells that John Gearhart and colleagues isolated from human fetal tissue in 1998. These cells can produce unchanged copies of themselves when maintained in culture, and they form into clumps called embryoid bodies. Under certain conditions, research has shown that the cells in the embryoid bodies begin to look and function like neurons when subjected to specific laboratory conditions [2]. The researchers had an idea that these embryoid body cells in their nonspecialized state might become specialized as replacement neurons if placed into the area of the damaged spinal cord. So they carefully prepared cells from the embryoid bodies and injected them into the fluid surrounding the spinal cord of the paralyzed rats that had their motor neurons destroyed by the Sindbis virus.

To test this idea, the researchers selected from laboratory culture dishes barely differentiated embryonic germs cells that displayed the molecular markers of neural stem cells, including the proteins nestin and neuron specific enolase. They grew these cells in large quantities and injected them into the fluid surrounding the spinal cords of partially paralyzed, Sindbis-virus-treated rats.

The response was impressive. Three months after the injections, many of the treated rats were able to move their hind limbs and walk, albeit clumsily, while the rats that did not receive cell injections remained paralyzed. Moreover, at autopsy the researchers found that cells derived from human embryonic germ cells had migrated throughout the spinal fluid and continued to develop, displaying both the shape and molecular markers characteristic of mature motor neurons. The researchers are quick to caution that their results are preliminary, and that they do not know for certain whether the treatment helped the paralyzed rats because new neurons took the place of the old, or because trophic factors from the injected cells facilitated the recovery of the rats' remaining nerve cells and helped the rats improve in their ability to use their hind limbs. Nor do they know how well this strategy will translate into a therapy for human neurodegenerative diseases like ALS. And they emphasize that there are many hurdles to cross before the use of stem cells to repair damaged motor neurons in patients can be considered. Nevertheless, researchers are excited about these results, which, if confirmed, would represent a major step toward using specialized stem cells from embryonic and fetal tissue sources to restore nervous system function.

REFERENCES

1. Kerr, D.A., Llado, J., Shamblott, M., Maragakis, N., Irani, D.N., Dike, S., Sappington, A., Gearhart, J., and Rothstein, J. (2001). Human embryonic germ cell derivatives facillitate motor recovery of rats with diffuse motor neuron injury.

2. Shamblott, M.J., Axelman, J., Wang, S., Bugg, E.M., Littlefield, J.W., Donovan, P.J., Blumenthal, P.D., Huggins, G.R., and Gearhart, J.D. (1998). Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc. Natl. Acad. Sci. U. S. A. *95*, 13726-13731.

MULTIPLE APPROACHES FOR USING STEM CELLS IN PARKINSON'S DISEASE RESEARCH

Efforts to develop stem cell based therapies for Parkinson's Disease provide a good example of research aimed at rebuilding the central nervous system. As is the case with other disorders, both the cell-implantation and the trophic-factor strategies are under active development. Both approaches are promising. This is especially true of cell implantation, which involves using primary tissue transplanted directly form developing fetal brain tissue. Parkinson's is a progressive movement disorder that usually strikes

after age 50. Symptoms often begin with an uncontrollable hand tremor, followed by increasing rigidity, difficulty walking, and trouble initiating voluntary movement. The symptoms result from the death of a particular set of neurons deep in the brain.

The neurons that die in Parkinson's Disease connect a structure in the brain called the substantia nigra to another structure called the striatum, composed of the caudate nucleus and the putamen (see Figure 8.2. Neuronal Pathways that Degenerate in Parkinson's Disease). These "nigro-striatal" neurons release the chemical transmitter dopamine onto their target neurons in the striatum. One of dopamine's major roles is to regulate the nerves that control body movement. As these cells die, there is less dopamine produced, leading to the movement difficulties characteristic of Parkinson's. At this point, no one knows for certain why the neurons die.

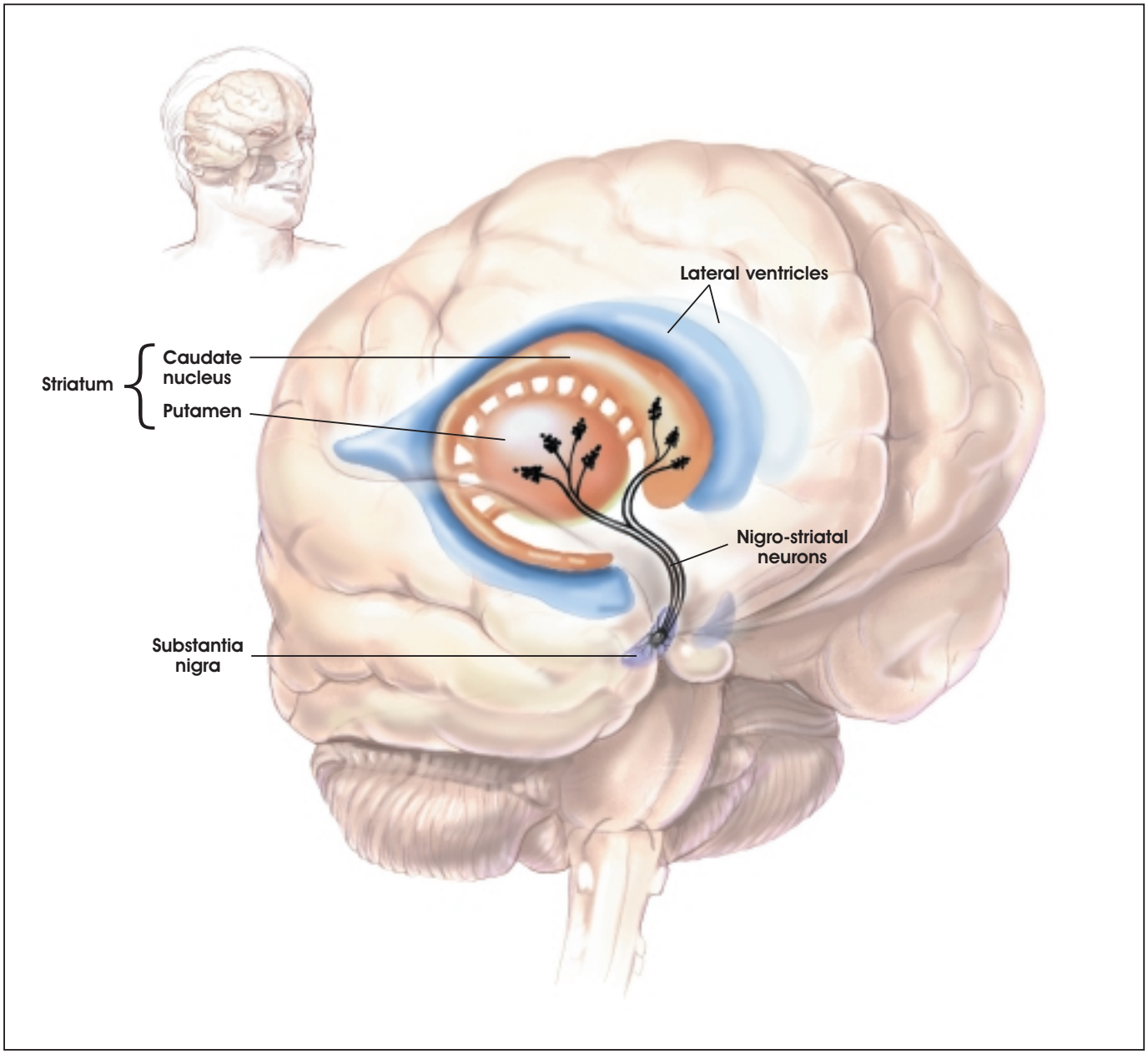


Figure 8.2. Neuronal Pathways that Degenerate in Parkinson's Disease.
Signals that control body movements travel along neurons that project from the substantia nigra to the caudate nucleus and putamen (collectively called the striatum). These "nigro-striatal" neurons release dopamine at their stargets in the striatum. In Parkinson's patients, dopamine neurons in the nigro-striatal pathway degenerate for unknown reasons.

Most patients suffering from Parkinson's Disease are treated with a drug called levodopa, which the brain converts into dopamine. It initially helps most patients, but unfortunately, side effects of the drug increase over time and its effectiveness wanes. This leaves Parkinson's patients and their doctors fighting a long, uphill battle to balance medication with side effects to maintain function. In the end, many patients are utterly helpless.

FETAL TISSUE TRANSPLANTS IN PARKINSON'S DISEASE RESEARCH

The idea of growing dopamine cells in the laboratory to treat Parkinson's is the most recent step in the long history of cell or tissue transplantation to reverse this devastating disease. The concept was, and still is, straightforward: implant cells into the brain that can replace the lost dopamine-releasing neurons. Although conceptually straightforward, this is not an easy task. Fully developed and differentiated dopamine neurons do not survive transplantation, so direct transplantation of fully developed brain tissue from cadavers, for example, is not an option. Moreover, full functional recovery depends on more than cell survival and dopamine release; transplanted cells must also make appropriate connections with their normal target neurons in the striatum.

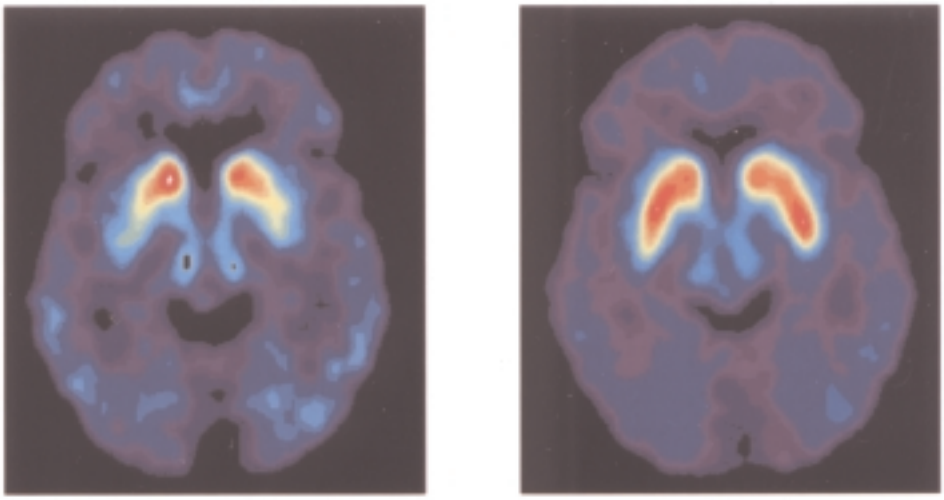
One of the first attempts at using cell transplantation in humans was tried in the 1980s. This surgical approach involved the transplantation of dopamine-producing cells found in the adrenal glands, which sit atop the kidneys in the abdomen. Neurosurgeons in Mexico reported that they had achieved dramatic improvement in Parkinson's patients by transplanting dopamine-producing chromaffin cells from several patients' own adrenal glands to the nigro-striatal area of their brains. Surgeons in the United States, however, observed only very modest and inconsistent improvement in their patients' symptoms, and any gains disappeared within a year after surgery. Furthermore, it became clear that the risks associated with the procedure—which required both brain and abdominal surgery on patients who are often frail and elderly—outweigh the benefits [13].

Another strategy, based on transplanting developing dopamine neurons from fetal brain tissue, has fared better, however. Lars Olsen and his colleagues showed in the early 1970s that fetal tissue transplanted directly from the developing nigro-striatal pathways of embryonic mice into the anterior chamber of an adult rat's eye continues to mature into fully developed dopamine neurons [3]. By the early 1980s Anders Bjorkland and others had shown that transplantation of fetal tissue into the damaged areas of the brains of rats and monkeys used as models of the disease could reverse their Parkinson's-like symptoms. Subsequently, researchers refined their surgical techniques and showed that functional recovery depends on the implanted neurons growing and making functional connections at the appropriate brain locations—essentially finishing their maturation by integrating into the adult host brain [3].

The promising animal results led to human trials in several centers worldwide, starting in the mid-1980s. Using tissue removed from a fetus electively aborted seven to nine weeks after conception, these early human transplantation studies showed encouraging, but inconsistent, benefit to patients. Although not all patients improved, in the best cases patients receiving fetal tissue transplants showed a clear reduction in the severity of their symptoms. Also, researchers could measure an increase in dopamine neuron function in the striatum of these patients by using a brain-imaging method called positron emission tomography (PET) (see Figure 8.3. Positron Emission Tomography [PET] images from a Parkinson's patient before and after fetal tissue transplantation). Also, autopsies done on the few patients who died from causes unrelated to either Parkinson's or the surgery revealed a robust survival of the grafted neurons. Moreover, the grafted neurons sent outgrowths from the cell body that integrated well into the normal target areas in the striatum.

A major weakness in these initial studies was that they were all done "open label," meaning that both researchers and patients knew which patients received the transplanted tissue. When appropriate, the best test of a new therapy is a placebo controlled, double-blind trial, in which neither researcher nor patient knows who has received the experimental treatment. In the mid-1990s, NIH approved funding for two rigorous clinical trials of fetal tissue transplantation for Parkinson's patients. Both studies provided for placebo control, in the form of sham surgery conducted on half the study patients, and they were done double blind—neither the

Dopamine-Neuron Transplantation



Before Surgery **After Surgery**

Figure 8.3. Positron Emission Tomography (PET) images from a Parkinson's patient before and after fetal tissue transplantation. The image taken before surgery (left) shows uptake of a radioactive form of dopamine (red) only in the caudate nucleus, indicating that dopamine neurons have degenerated. Twelve months after surgery, an image from the same patient (right) reveals increased dopamine function, especially in the putamen. (Reprinted with permission from N Eng J Med 2001;344 (10) p. 710.)

researchers evaluating the effects of the surgery nor the patients themselves knew who got tissue implants.

The results of one of these trials, led by Curt Freed, were published recently [5]. Compared with control, patients who received the fetal-tissue transplant showed no significant benefit in a subjective assessment of the patient's quality of life, which was the study's primary endpoint. Moreover, two years after surgery, 5 out of 33 treated patients developed persistent dyskinesia—uncontrolled flailing movements—that had not been observed in the open-label work described above.

The Freed study results, nonetheless, provide important information about the ability of dopamine neurons to survive in humans. Moreover, PET-scanning data from the treated patients, as well as autopsies of two patients who died of unrelated causes several months after the surgery, showed that many of the dopamine neurons survived and grew. Researchers are now awaiting the results of the second NIH-sponsored double-blind trial, led by Warren Olanow [12]. The procedures used in this study differ substantially from those of Freed and his colleagues—including the tissue-handling method, the number of cells implanted, the use of immunosuppressive drugs to limit rejection of the implanted tissue, and the tests used to assess patient response—and are closer to those used in the most successful of the early open-label experiments.

Most Parkinson's researchers are still hopeful that the cell-implantation approach will one day lead to a useful and widely used therapy for Parkinson's Disease. At the same time, however, most researchers are also convinced they must find a different source of cells for transplant. The logistical and technical problems involved in recovering enough developing dopamine neurons from fetal tissue are very great. Moreover, it is virtually impossible to standardize the tissue collected from different fetuses and to fully characterize the cells implanted. This absence of tissue standardization makes it very difficult to determine the most important factors that lead to a good patient response and may add risk (see Chapter 10. Assessing Human Stem Cell Safety).

One alternative to cell implantation with human fetal tissue is to use fetal cells and tissues from animals. Researchers at Diacrin and Genzyme, two biotechnology companies, recently announced preliminary results from a clinical trial in which 10 Parkinson's patients received neural cells from the

brains of fetal pigs. Eighteen months after the surgery, treated patients did not improve enough to show a statistically significant difference from eight control patients who received a sham immunosuppression regimen and underwent sham surgery. Autopsy of one treated patient who died of a pulmonary embolism eight months after surgery revealed that a small portion of the transplanted pig cells had survived [2], but PET studies looking for improvement in dopamine uptake in all treated patients did not show clear improvement. The researchers are still analyzing their data [15].

RAISING NEURONS FOR REPLACEMENT IN PATIENTS WITH PARKINSON'S DISEASE

What Parkinson's researchers ultimately want is a renewable source of cells that can differentiate into functional dopamine neurons when placed in the striatum. Laboratory-grown cells derived from a stem cell may be the best potential alternative source for transplantable material. One way to get these is to find the right combination of growth factors and cell-culture conditions to bring undifferentiated cells along in a culture dish to a point where they are committed to becoming dopamine neurons, then implant them to finish growth and differentiation in the host brain. Another possibility is to put less-committed cells into a damaged brain and rely on "environmental" signals in the brain to guide them into becoming the right kind of replacement cell. These developmental signals may be expressed in the brain transiently following neural degeneration or acute damage.

Whether the cells ultimately implanted are half-differentiated or completely immature, however, researchers need a reliable source. To that end, they have identified a whole host of different immature cells that may have the potential to become, among other things, dopamine neurons, and they are now in the process of sorting out how best to make them do so. Neural stem cells isolated from animals and humans cannot be grown efficiently in the lab without changing them in some way, such as by engineering them to express a gene normally turned on only early in development. Embryonic stem cells—derived from the inner cell mass of an embryo at the blastocyst stage, when only a few hundred cells are present—can be kept in culture in a completely undifferentiated state. They are still capable of becoming not just nervous system cells but every cell type in the body. If researchers want to be able to implant cells derived from undifferentiated embryonic stem cells, they must take care that no cells in the mix give rise to unwanted cell types, such as muscle or bone, within the nervous system. Stem cells from other tissues—including umbilical cord blood and human bone marrow—can also be coaxed to display many of the surface-protein "markers" characteristic of nervous system cells. It is not yet clear, however, whether these cells are capable of giving rise to fully functional neurons.

A great deal of basic research remains to be done to find which of these cells provides the best way to get a workable therapy for Parkinson's Disease. For example, although researchers have shown for certain that both primary human fetal cells and mouse embryonic stem cells can become fully functional dopamine neurons, they do not yet know if adult neural stem cells have the same potential. Also, no one has yet published evidence that cells from any renewable source that are laboratory-directed to differentiate into dopamine neurons can eliminate symptoms in animal models of Parkinson's when implanted.

Researchers are making rapid progress, however. For example, Ron McKay and his colleagues at NIH reported in 1998 that they were able to expand a population of neurons from embryonic mouse brain in culture, and that these cells relieved Parkinson's-like symptoms in a rat model [16]. And last year, McKay's lab also described a procedure for efficiently converting mouse pluripotent embryonic stem cells into neurons that have all the characteristics of dopamine neurons, including the ability to form synapses [17]. McKay and other researchers say they have encouraging unpublished results that dopamine precursors derived from mouse embryonic stem cells can eliminate symptoms in rat models of Parkinson's Disease [7, 10].

Privately funded researchers are following an analogous path using pluripotent human embryonic stem cells. Thomas Okarma of Geron Corporation confirms that his company is testing the potential of human embryonic stem cells in animal models of Parkinson's Disease, but the results are not yet complete [11]. In abstracts presented at a recent conference, Geron reports having succeeded in directing human embryonic stem cells to become mature neural cells

in laboratory culture, including cells that have the structural and chemical characteristics of dopamine neurons [6].

TURNING ON THE BRAIN'S OWN STEM CELLS AS A REPAIR MECHANISM

Parkinson's researchers are also looking for ways to spark the repair mechanisms already in a patient's brain to fix damage that these mechanisms could not otherwise manage. This strategy is less developed than cell implantation, but it also holds promise [1]. In the future, researchers may use stem cells from embryonic or adult sources not to replace lost cells directly, but rather to turn on the body's own repair mechanisms. Alternatively, researchers may find effective drug treatments that help a patient's own stem cells and repair mechanisms work more effectively.

Stem cells in the adult primate brain occur in two locations. One, the subventricular zone, is an area under fluid-filled spaces called ventricles. The other is the dentate gyrus of the hippocampus. In primates, very few new neurons normally appear in either place, which is why the phenomenon escaped notice until recently. Researchers showed in the mid-1990s that when the brain is injured, stem cells in these two areas proliferate and migrate toward the site of the damage. The researchers are now trying to discover how far this kind of response can go toward ameliorating certain kinds of damage.

Recent research shows the direction that this may be heading for Parkinson's Disease. James Fallon and colleagues studied the effects on rat brain of a protein called transforming growth factor alpha (TGFα)—a natural peptide found in the body from the very earliest stages of embryonic development onward that is important in activating normal repair processes in several organs, including liver and skin. Fallon's studies suggest that the brain's normal repair process may never be adequately triggered in a slowly developing degenerative disease like Parkinson's and that providing more TGFα can turn it on. Specifically, Fallon found that TGFα injected into healthy rat brain causes stem cells in the subventricular zone to proliferate for several days, after which they disappear. But if the researchers make similar injections into rats in which they first damage the nigro-striatal neurons with a toxin called 6-hydroxydopamine—a frequently used animal model for Parkinson's Disease—two things happen. After several days of cell proliferation, Fallon observes what he calls a "wave of migration" of the stem cells to the damaged areas, where they differentiate into dopamine neurons. Most importantly, the treated rats do not show the behavioral abnormalities associated with the loss of the neurons. Whether the beneficial effect on symptoms is the result of the newly formed cells or some other trophic effect is not yet entirely clear [4].

STEM CELLS' FUTURE ROLE IN SPINAL CORD INJURY REPAIR

Parkinson's Disease is only one of many nervous system disorders that researchers are trying to solve by regenerating damaged tissue. But Parkinson's, difficult as it is to reverse, is a relatively easy target because a regenerative therapy need only replace one particular cell type in one part of the brain.

Therapies for other disorders face much bigger hurdles. Complete restoration after severe spinal cord injury, for example, is probably far in the future, if it can ever be done at all. Many cell types are destroyed in these injuries, including neurons that carry messages between the brain and the rest of the body. Getting these neurons to grow past an injury site and connect appropriately with their targets is extraordinarily difficult. But spinal cord injury patients would benefit greatly from an even limited restoration of lost functions—gaining partial use of a limb instead of none, or restoring bladder control, or being freed from pain. Such limited restoration of part of a patient's lost functions is, for some less severe types of injury, perhaps a more achievable goal.

In many spinal injuries, the spinal cord is not actually cut and at least some of the signal-carrying neuronal axons are intact. But the surviving axons no longer carry messages because cells called oligodendrocytes, which make the axons' insulating myelin sheath, are lost. Researchers have recently made the first steps in learning to replace these lost myelin-producing cells [14]. For example, researchers have shown that stem cells can aid remyelination in rodents [8, 9]. Specifically, they found that injection of oligodendrocytes derived from mouse embryonic stem cells could remyelinate axons in chemically demyelinated rat spinal cord and that the treated

rats regained limited use of their hind limbs compared with the controls. They are not certain, however, whether the limited increase in function they observed in rats is actually due to the remyelination or an unidentified trophic effect of the treatment.

Spinal injury researchers emphasize that much more basic and preclinical research must be done before attempting human trials using stem cell therapies to repair the damaged nervous system. Despite the fact that there is much basic work left to do and many fundamental questions still to be answered, researchers are hopeful that effective repair for once-hopeless nervous system damage may eventually be achieved. Whether through developing replacement cells or activating the body's own stem cells *in vivo*, research on the use of stem cells for nervous system disorders is a rapidly advancing field. This research promises to answer key questions about how to repair nervous system damage and how to restore key body functions damaged by disease or disability.

REFERENCES

1. Bjorklund, A. and Lindvall, O. (2000). Self-repair in the brain. Nature. *405*, 892-895.

2. Deacon, T., Schumacher, J., Dinsmore, J., Thomas, C., Palmer, P., Kott, S., Edge, A., Penney, D., Kassissieh, S., Dempsey, P., and Isacson, O. (1997). Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nat. Med. *3*, 350-353.

3. Dunnett, S.B., Bjorklund, A., and Lindvall, O. (2001). Cell therapy in Parkinson's disease—stop or go? Nat. Rev. Neurosci. *2*, 365-369.

4. Fallon, J., Reid, S., Kinyamu, R., Opole, I., Opole, R., Baratta, J., Korc, M., Endo, T.L., Duong, A., Nguyen, G., Karkehabadhi, M., Twardzik, D., and Loughlin, S. (2000). *In vivo* induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. Proc. Natl. Acad. Sci. U. S. A. *97*, 14686-14691.

5. Freed, C.R., Greene, P.E., Breeze, R.E., Tsai, W.Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Trojanowski, J.Q., Eidelberg, D., and Fahn, S. (2001). Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N. Engl. J. Med. *344*, 710-719.

6. Harley, C.B., Gearhart, J., Jaenisch, R., Rossant, J., and Thomson, J. (2001). Pluripotent stem cells: biology and applications. Durango, CO.

7. Isacson, O., personal communication.

8. Liu, S., Qu, Y., Stewart, T.J., Howard, M.J., Chakrabortty, S., Holekamp, T.F., and McDonald, J.W. (2000). Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc. Natl. Acad. Sci. U. S. A. *97*, 6126-6131.

9. McDonald, J.W., Liu, X.Z., Qu, Y., Liu, S., Mickey, S.K., Turetsky, D., Gottlieb, D.I., and Choi, D.W. (1999). Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat. Med. *5*, 1410-1412.

10. McKay, R., personal communication.

11. Okarma, T., personal communication.

12. Olanow, C. W., personal communication.

13. Quinn, N.P. (1990). The clinical application of cell grafting techniques in patients with Parkinson's disease. Prog. Brain Res. *82*, 619-625.

14. Raisman, G. (2001). Olfactory ensheathing cells—another miracle cure for spinal cord injury? Nat. Rev. Neurosci. *2*, 369-374.

15. Schumacher, J.M., Ellias, S.A., Palmer, E.P., Kott, H.S., Dinsmore, J., Dempsey, P.K., Fischman, A.J., Thomas, C., Feldman, R.G., Kassissieh, S., Raineri, R., Manhart, C., Penney, D., Fink, J.S., and Isacson, O. (2000). Transplantation of embryonic porcine mesencephalic tissue in patients with PD. Neurology. *54*, 1042-1050.

16. Studer, L., Csete, M., Lee, S.H., Kabbani, N., Walikonis, J., Wold, B., and McKay, R. (2000). Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. J. Neurosci. *20*, 7377-7383.

17. Studer, L., Tabar, V., and McKay, R.D. (1998). Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. Nat. Neurosci. *1*, 290-295.

This page intentionally left blank